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A New Hybrid *Polypodium* Provides Insights Concerning the Systematics of *Polypodium scouleri* and its Sympatric Congeners

T. J. Hildebrand, Christopher H. Haufler, James P. Therrien, and Cathy Walters

Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, Kansas 66045-2106

Philip Hammond

University Herbarium, University of California, Berkeley, CA 94720

Abstract.—With its thick, leathery leaves, reticulate venation, and large sori, *Polypodium scouleri*, located in a narrow band along the Pacific coast of North America, is the most distinctive member of the cosmopolitan *P. vulgare* species complex. Although early studies based on morphology and chromosomes yielded hypotheses about the relationships among some elements of this complex, phylogenetic alliances to *P. scouleri* were not proposed. Combining data from *rbcL* and *trnL* DNA sequences with isozymic analyses suggested that *P. scouleri* originated relatively recently and is closely allied to and sympatric with *P. californicum* and *P. glycyrrhiza*. Consistent with a hypothesis of recent origin, we detected no infraspecific isozymic variation across the range of *P. scouleri*. Although allopolyploidy is a common feature of the *P. vulgare* complex, *P. scouleri* stands out because it has not been implicated in the origin of any secondary (allotetraploid) species. However, as early as 1951, Manton reported a triploid individual that was morphologically similar to *P. scouleri*, but whose other parent could not be verified. Since that time, others have suggested that *P. scouleri* might be crossing with sympatric congeners, but no solid evidence has been obtained. The present study confirmed that *P. scouleri* hybridized with neighboring *P. calirhiza*, and showed that individuals with intermediate morphological features contained isozyme marker alleles from both parental lineages.

The *Polypodium vulgare* L. complex (Polypodiaceae) first drew attention with the publication of cytological counts for several European and North American members (Manton, 1950). Controlled crosses among members of the complex followed (Shivas, 1961), and resulted in further taxonomic studies founded on interbreeding boundaries and subtle morphological distinctions. A major organizational leap in the circumscription of North American *Polypodium* L. species was the definition of eastern and western complexes (Lloyd and Lang, 1964). In their search to identify parental lineages for allopolyploid members, Lloyd and Lang grouped the complexes on the presence (eastern) or absence (western) of sporangiasters. Although sporangiasters were later shown to constitute synapomorphies in several western species (*P. amorphum* Suksdorf, *P. saximontanum* Windham, and *P. sibiricum* Siplivinsky [Haufler and Windham, 1991]), recognizing the importance of these unique soral features within American polypodiums was insightful. Additionally, Lloyd and Lang (1964) hypothesized that the progenitors of the allotetraploid *P. californicum* Kaulfuss were *P. glycyrrhiza* D. C. Eaton (2*n*)

and diploid *P. californicum* Kaulfuss. It was not until much later, however, that Whitmore and Smith (1991) described the tetraploid cytotype as a distinct and reproductively competent species, *P. calirhiza* S. A. Whitmore & A. R. Smith.

Research explorations of morphology (Haufler and Windham, 1991; Whitmore and Smith, 1991; Haufler *et al.*, 1993), cytology (Haufler and Wang, 1991), isozyme variation (Haufler *et al.*, 1995b), chloroplast DNA restriction site analysis (Haufler *et al.*, 1995a), and DNA sequence data (Haufler and Ranker, 1995) have further defined relationships within the *Polypodium vulgare* complex. Yet a review of the above reveals the exclusion of *P. scouleri* Hooker & Greville from all but two studies (Haufler *et al.*, 1993; Haufler and Ranker, 1995).

Confined to a narrow distribution along the Pacific Coast of North America, and tentatively recognized as a member of the western complex, the distinctive morphology of *P. scouleri* clearly separating it from its congeners precluded the formulation of accurate hypotheses about phylogenetic relationships. The overview by Haufler *et al.* (1993, pp. 315–323) in their treatment of *Polypodium* in Flora of North America (Fig. 1) allied *P. scouleri* with other western members, and provided a detailed morphological description. In addition, the investigation of *rbcL* sequence data suggested a sister taxon relationship with *P. glycyrrhiza* that was supported by isozyme profiles (Haufler and Ranker, 1995). Haufler and Ranker (1995) hypothesized that the particularly distinctive morphological features of *P. scouleri* may have evolved through adaptive response to environmental stress. That study did not consider another close relative, *P. californicum*, and, because only diploids were included, did not incorporate allopolyploid *P. calirhiza*.

Recently, leaves resembling *P. scouleri* but having some atypical features were collected at a site in California. At the same time, leaves of *P. calirhiza* and more typical *P. scouleri* were obtained. The present study was designed to examine the atypical leaves morphologically and use molecular approaches to determine whether these plants originated through hybridization between *P. scouleri* and other members of the western complex. Several morphological features of suspected hybrid leaves were investigated and compared with features of typical *P. scouleri* and *P. calirhiza* leaves. Starch gel electrophoresis was used to reveal isozyme marker alleles and characterize the suspected hybrid and parental lineages.

Material and Methods

Study Area.—Located within the confines of highly populated San Francisco County are the natural vegetation preserves of Tank Hill and Mt. Sutro (Fig. 2). The Open Space Program of the San Francisco Recreation and Parks Department retains ownership of these sanctuaries. Land stewards manage vegetation of the preserves, emphasizing enhancement and restoration of native species as well as the removal of invasive exotic plant populations.

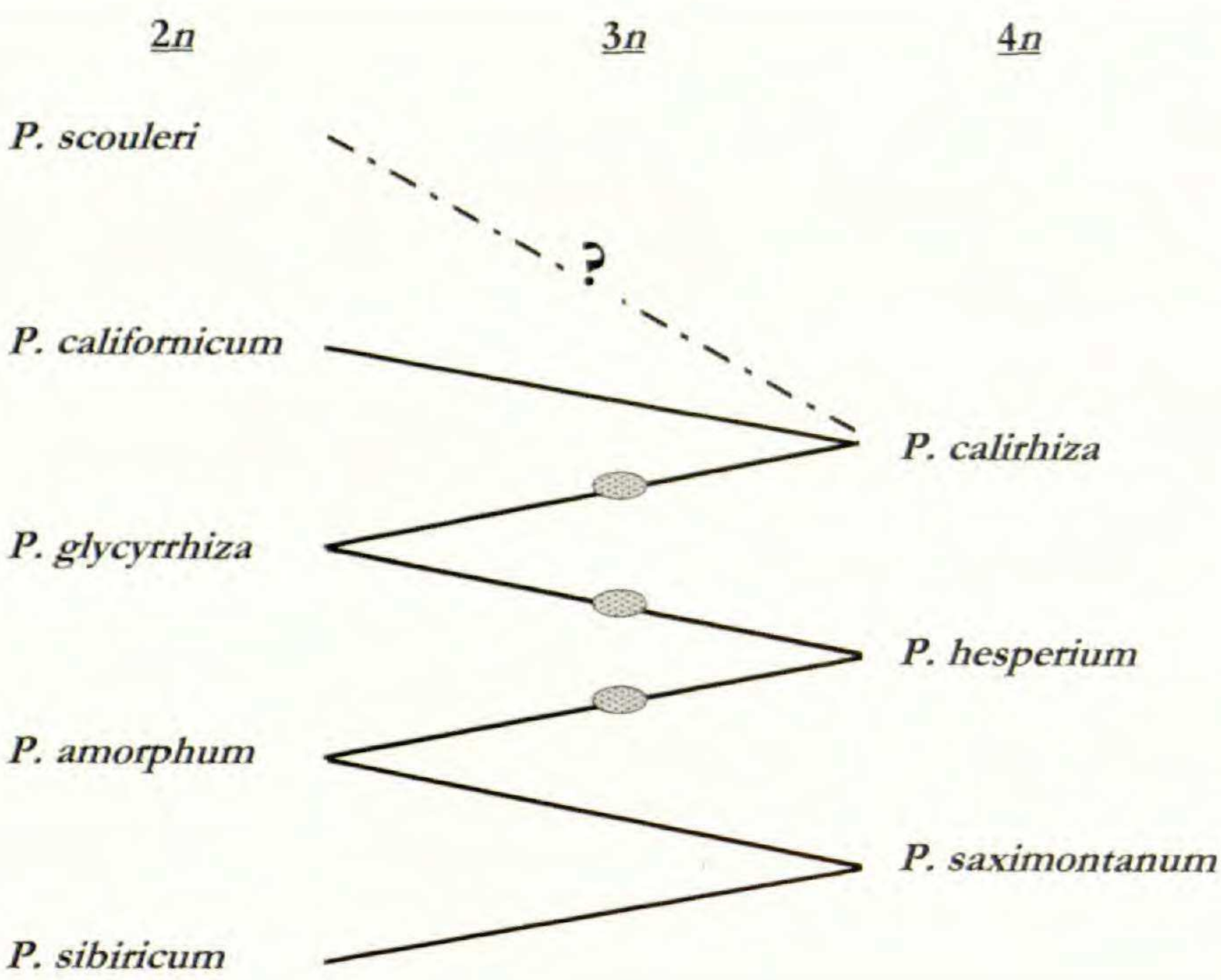

FIG. 1. Kinship of diploid and tetraploid members of the Western *Polypodium vulgare* complex from Haufler, *et al.* (1993). All taxa are restricted to western North America with the exception of *P. sibiricum*, which is circumboreally distributed in North America and Asia. Shaded ovals represent sterile backcrosses (3*n*) in the complex. The dashed line between *P. scouleri* and *P. calirhiza* indicates the subject of the present study.

A dense forest of mature, non-native cypress (*Cupressus macrocarpa* Hartw. Ex Gord.) and eucalyptus (*Eucalyptus globulus* Labill.) was planted on Mt. Sutro as early as 1870 and now covers much of the hill. *Polypodium* species on Mt. Sutro flourish beneath the forest canopy, nestled among rocks and at the bases of trees as hemiepiphytes. Leaves of *Polypodium calirhiza* and *P. scouleri* were collected from the east face of Mt. Sutro (Site 1, Fig. 2; Table 1).

Eucalyptus and cypress were also planted on Tank Hill, but, in contrast to Mt. Sutro, they are sporadic on Tank Hill, and primarily at lower elevations. The summit of Tank Hill is dominated by large tracts of rocky fields and ledges of Franciscan radiolarian chert. In the fields and on the ledges are *Polypodium* populations exposed to the harsh sun and buffeted by gusting winds. Native species associated with ferns in this predominantly open habitat include: Nootka reed grass (*Calamagrostis nutkaensis* (Presl) Steud.), yarrow (*Achillea millefolium* L.), coast barberry (*Berberis pinnata* Lag.), soap plant (*Chlorogalum pomeridianum* (DC.) Kunth. var. *divaricatum* (Lindl.) Hoov.), and seaside daisy (*Erigeron glaucus* Ker.). Among the boulders and crevices near the crest of Tank Hill, leaves of *Polypodium calirhiza* and

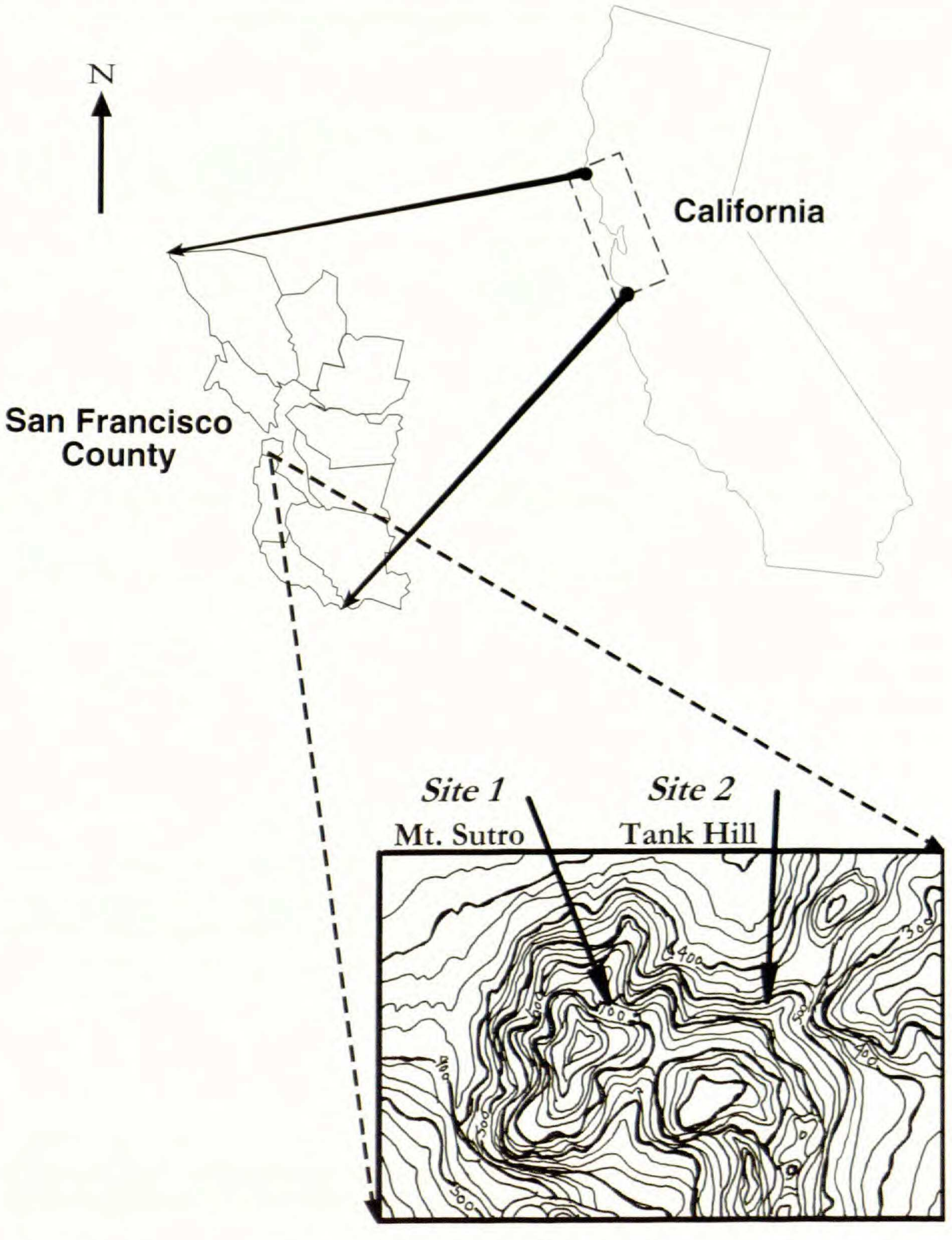

FIG. 2. Collection localities on Mt. Sutro and Tank Hill, San Francisco County, California.

P. scouleri, as well as the suspected hybrid, were collected (Site 2, Fig. 2; Table 1). To characterize the amount of electrophoretically detectable genetic variation across the range of *P. scouleri*, population samples were obtained from four additional California populations. Representative specimens of all

TABLE 1. California sites from which *Polypodium* plants were collected. All specimens are deposited in the McGregor Herbarium, University of Kansas (KANU). N = number of individuals for each population.

Collection Site (county)	Species (ploidy level: x = 37)	N	Voucher
Mt. Sutro (San Francisco)	*P. calirhiza* (4x)	5	*Hildebrand 3217*
Mt. Sutro (San Francisco)	*P. scouleri* (2x)	3	*Hildebrand 3216*
Tank Hill (San Francisco)	*P. calirhiza* (4x)	12	*Hildebrand 3219*
Tank Hill (San Francisco)	*P. scouleri* (2x)	7	*Hildebrand 3218*
Tank Hill (San Francisco)	*P. calirhiza* × *scouleri* (?)	5	*Hildebrand 3220 & 3221*
Fern Canyon (Trinity)	*P. scouleri* (2x)	20	*Therrien s.n.*
Point Reyes National Seashore (Marin)	*P. scouleri* (2x)	15	*Therrien s.n.*
Trinidad (Humboldt)	*P. scouleri* (2x)	6	*Therrien s.n.*
Fort Ross (Sonoma)	*P. scouleri* (2x)	3	*Therrien s.n.*

collections (Table 1) were pressed and deposited at McGregor Herbarium of the University of Kansas (KANU).

METHODS.—Leaves were removed from live material in the field, placed in plastic bags, and shipped on ice. Upon arrival, bags were transferred to 4°C storage where they were kept until preparation for electrophoresis. At least one sample of plant material for each leaf was prepared immediately upon receipt in the laboratory. Most leaves, particularly of *P. scouleri* and the suspected hybrid, retained their fresh appearance in storage for considerable time (up to one month). Preparations from fresh plant material stored for longer periods yielded banding patterns comparable to that prepared immediately, indicating extended retention of enzymatic activity.

Plant material was prepared by crushing in phosphate-PVP buffer (Soltis *et al.*, 1983) followed by absorption of the homogenate into filter paper wicks and storage of the wicks at −80° C. Freezing prepared fresh material allowed storage of samples for several months with no loss of enzymatic activity.

Selection of enzymes and the systems best suited for their survey was based on previous studies of *Polypodium* (Haufler *et al.*, 1995a, 1995b) and other fern genera (Haufler, 1985b; Haufler *et al.*, 1990; Soltis *et al.*, 1990; Pryer and Haufler, 1993). Banding patterns were obtained by electrophoresis on 12.4% starch gels for the following enzymes: aldolase (ALD), fructose 1,6-bisphosphatase (FBP), glyceraldehyde 3-phosphate dehydrogenase (G-3PDH), hexokinase (HK), isocitrate dehydrogenase (IDH), phosphogluconate dehydrogenase (PGDH), triosephosphate isomerase (TPI), and phosphoglucoisomerase (PGI). Bands were resolved best for ALD and IDH on system 11 (Haufler, 1985b) whereas only the 7.5 pH version of the morpholine/citrate system (MC) (Clayton and Tretiak, 1972) revealed clear bands for G-3PDH. Both system 11 and MC resolved bands for FBP, MC and system 8 (Haufler, 1985a) for MDH, and MC and system 6 (Soltis *et al.*, 1983) for PGDH. System 8 also revealed bands for HK, LAP, and, in addition to system 6, for PGI. TPI bands were revealed only with system 6. Digital images were obtained of all

TABLE 2. Comparisons of character states identified for *Polypodium scouleri*, *calirhiza* × *scouleri*, and *P. calrhiza*.

Character	*Polypodium scouleri*	*Polypodium calirhiza* × *scouleri*	*Polypodium calirhiza*
Frond texture	stiff, leathery	stiff, leathery	herbaceous
Rhacis scales	broadly ovate, tapering to ca 3 cells in width; not occurring in pairs	narrowly ovate to broadly lanceolate; often adjacent and fused (from the base) to one third their length	lanceolate to lanceolate-ovate; 3–6 cells wide, tapering to 1–3 cells; not occurring in pairs
Pinna venation	regularly anastomosing, forming one row of areoles	mixed, primarily free but occasionally anastomosing and forming areoles	free, no areoles formed
Guard cells			
- shape	round	round	elliptic
- mean length: μm (range)	35 (33–38)	38 (35–43)	56 (48–63)
Subsidiary cell margins	smooth	distinctly lobed	distinctly lobed
Approximate stomata density	150/mm^2	70/mm^2	40/mm^2
Spore length: μm (s.d.)	61.3 (± 8.4)	malformed	61.5 (± 7.1)
Sori			
- shape	round or oblong; shape specific to individual plant	round or oblong; both shapes on individual plants	oblong
- distribution	closest to midrib	variable	midway between costa and margin
- size	generally > 3 mm	variable, 1–4 mm, to absent	generally < 3 mm

gels using a Nikon CoolPix 950 camera and visualized with Adobe Photoshop 5.5 software for Macintosh.

Morphological characters documented as useful for delimiting *Polypodium* species (Haufler *et al.*, 1993; Whitmore and Smith, 1991) were examined on leaves from both parental species and the hybrid. These included leaf texture, sorus diameter, pinna venation, spore length, and rachis scale width. In addition, lower surface (abaxial) epidermal peels were produced to measure sizes and characterize shapes of guard and subsidiary cells, as well as differences in stomatal density (Table 2).

RESULTS

MORPHOLOGY.—The leaves of *Polypodium scouleri* differ from those of other members of the genus (although leaves of *P. calirhiza* may become somewhat thickened in exposed coastal environments) by their leathery texture, and

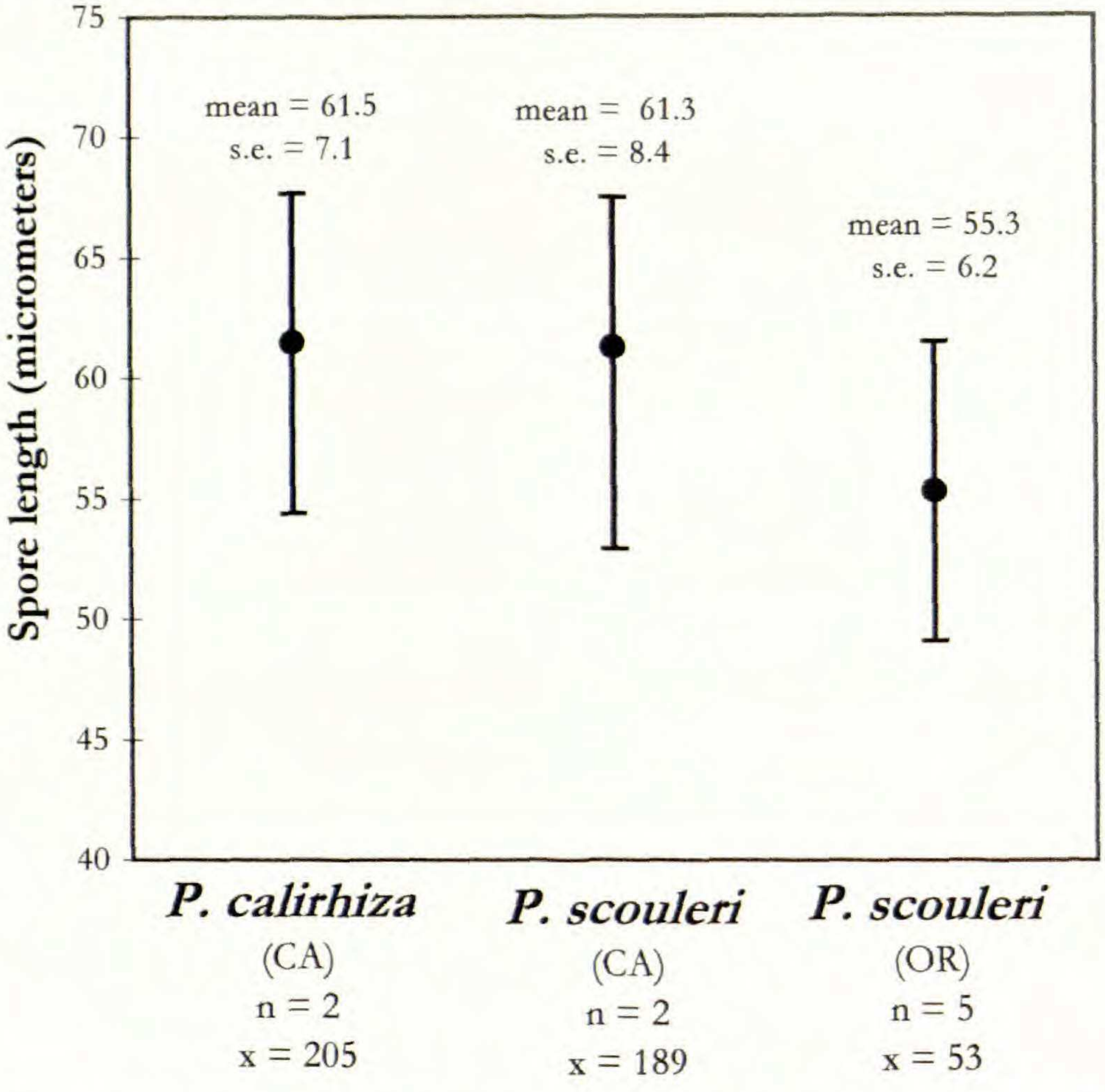

FIG. 3. Comparisons of spore length from plants gathered at the Mt. Sutro and Tank Hill *Polypodium* populations, and from a *P. scouleri* population from Oregon. Vertical bars represent +/− one standard error. Expected mean spore lengths (Haufler *et al.*, 1993) for *P. calirhiza* and *P. scouleri* are > 58 μm and < 53 μm, respectively. n = number of plants sampled; x = number of spores measured.

their well-defined aeroles produced by anastomosing venation. Other distinctive morphological features include individual leaf segments that are greater than 12 mm in width, soral diameters of more than 3 mm, and rachis scales that are large, pale reddish brown and broadly triangular, tapering to a point less than three cells wide (Table 2). The pair of guard cells surrounding the stomata on the lower epidermal surface is circular in outline; individual guard cells average 35 μm in length, and are adjoined by smooth-margined subsidiary cells. Stomata density is approximately 150 stomata/μm^2. Tank Hill and Mt. Sutro *P. scouleri* populations had a mean spore length of 61.3 μm (Fig. 3).

Polypodium calirhiza plants have herbaceous leaves lacking the leathery texture of *P. scouleri* and are, in general, of smaller stature. Pinna venation is free or weakly anastomosing with some to many segments lacking aeroles. Leaf segments seldom exceed 12 mm wide, and soral diameters are less than 3 mm. Rachis scales, as in *P. scouleri*, are a translucent, pale reddish brown,

but, in contrast to *P. scouleri*, are lanceolate, only a few cells wide proximally, and narrow to only one to three cells distally. Epidermal peels showed elliptic paired guard cells (average length: 56 μm) and subsidiary cells with distinctly lobed margins. In striking contrast to that observed for *P. scouleri* stomata, stomata density is approximately 40 stomata/mm^2. Spores of the California *P. calirhiza* plants averaged a mean length of 61.5 μm.

The putative hybrid individuals showed both hybrid vigor (Charlesworth and Charlesworth, 1987) and dwarfing of leaves. On nearby Mt. Davidson, a more sheltered locale, *P. scouleri* produced leaves greater than 70 cm in length and lush in appearance. *Polypodium calirhiza* morphotypes vary with exposure, and increasingly open areas produce plants of more diminutive stature.

Morphological character states observed for hybrid leaves were either i) a combination of discrete features inherited without change from each parent, or ii) an additive blending of parental traits resulting in intermediate morphological states (Table 2). An exception was the consistently leathery, stiff texture of hybrid leaves, a phenotype that resembles only the *P. scouleri* parent. Leaves of the hybrid never had the herbaceous texture of the *P. calirhiza* lineage.

Pinna venation of hybrid leaves incorporates features of both parents: most veins are free, but occasional anastomoses and areoles occur. Soral size and development are extremely variable on hybrid leaves and of three general categories. Sori were 1) as large or slightly larger than those typical of *P. scouleri* (> 3 mm), 2) smaller and resembling *P. calirhiza* sori (< 3 mm), or 3) entirely undeveloped. Translucent, reddish brown scales occur along the rachis of hybrid leaves, but, in contrast to the deltate and lanceolate parental scales (*P. scouleri* and *P. calirhiza*, respectively), rachis scales on hybrid leaves are narrowly triangular. In addition, adjacent rachis scales are often fused (from the base) for approximately one third their length. Paired guard cells were orbicular in outline, as observed for *P. scouleri*, whereas subsidiary cells showed the distinctive, lobed margins of *P. calirhiza*. The average length of guard cells (38 μm), and stomatal densities of approximately 70 stomata/mm^2 are intermediate between parental lineages. All spores on hybrid plants were shrunken and malformed, suggesting inviability.

MOLECULAR.—Previous isozymic work by Haufler *et al.* (1995b) investigated *P. californicum* and *P. glycyrrhiza* (the progenitors of *P. calirhiza*) and identified electrophoretic markers for each diploid member, based on sampling the infraspecific variation from eleven populations. In the present study, isozyme profiles of the Tank Hill and Mt. Sutro individuals of *P. scouleri* were verified as representative for the species by sampling other populations (Table 1). In contrast to the genetic variability detected for other *Polypodium* diploids, *P. scouleri* isozymes were monomorphic across all populations and for all enzymes sampled. Of the ten enzyme systems considered, four (HK, PGI, PGDH, and MDH) yielded reproducible, well resolved banding patterns

that discriminated individuals representing parental species and hybrids. Allozymes (allelic variants within loci) are distinguished from isozymes (products from different loci) by assuming that models of gene product compartmentalization (Gastony and Darrow, 1983) are applicable to *Polypodium* enzymes.

The following results were obtained for each of the applicable enzyme systems. Gels stained for the monomeric enzyme hexokinase (HK) showed a slow-migrating allele in *P. calirhiza* samples in contrast to the faster allele present in *P. scouleri*. The hybrid expressed both alleles, one from each parental species (Fig. 4a). Two isozyme loci were resolved for phosphoglucoisomerase (PGI) and phosphogluconate dehydrogenase (PGDH). The faster migrating isozymes (*Pgi* 1, *Pgdh* 1) for both enzymes were monomorphic for all samples investigated. In contrast, banding patterns for the slower migrating isozymes (*Pgi* 2, *Pgdh* 2) were more variable for each enzyme (Fig. 4b & 4d). *Polypodium calirhiza* possessed a slower migrating allozyme for *Pgi* 2, whereas *P. scouleri* revealed a faster allozyme. The hybrid exhibited an additive banding pattern for dimeric phosphoglucoisomerase, possessing both allelic variants (Fig. 4b). A similar pattern was observed from gels stained for phosphogluconate dehydrogenase (PGDH). The polymorphic locus (*Pgdh* 2) expressed only the faster migrating allele in *P. calirhiza*, both allelic variants in *P. scouleri*, and an additive banding pattern in the hybrid (Fig. 4d). Three isozymes were revealed for malate dehydrogenase of which two (*Mdh* 2, *Mdh* 3) were monomorphic across all samples. The polymorphic locus (*Mdh* 1) produced bands that represent a fast allele for *P. calirhiza* and a slower migrating allele present in *P. scouleri* samples. In addition, both intra- and inter-locus heterodimers were formed during electrophoresis and were subsequently revealed by staining for malate dehydrogenase. Bands produced by the formation of both intra- and inter-locus heterodimers further supported the hybrid pattern of additive banding from parental species (Fig. 4c).

Discussion

Hybridization between species is a frequent phenomenon in plants and is especially common in pteridophytes (Wagner, 1968). The clarity and precision of species recognition and the accuracy of phylogenetic hypotheses can be enhanced by identifying and characterizing naturally occurring hybrids. Especially in groups such as the *Polypodium vulgare* complex, where species differences are particularly subtle, unrecognized interspecific hybrids that usually blend features of the parental individuals can appear to bridge gaps between otherwise distinct species.

Zymograms were exceptionally informative for delimiting *P. scouleri* and *P. calirhiza*, and for unequivocal verification of hybrid leaves collected on Tank Hill and Mt. Sutro. Typically, additive banding patterns indicate the presence of both parental alleles and are observed in hybrids (Crawford, 1990; Murphy *et al.*, 1996). The additive banding patterns visualized on gels

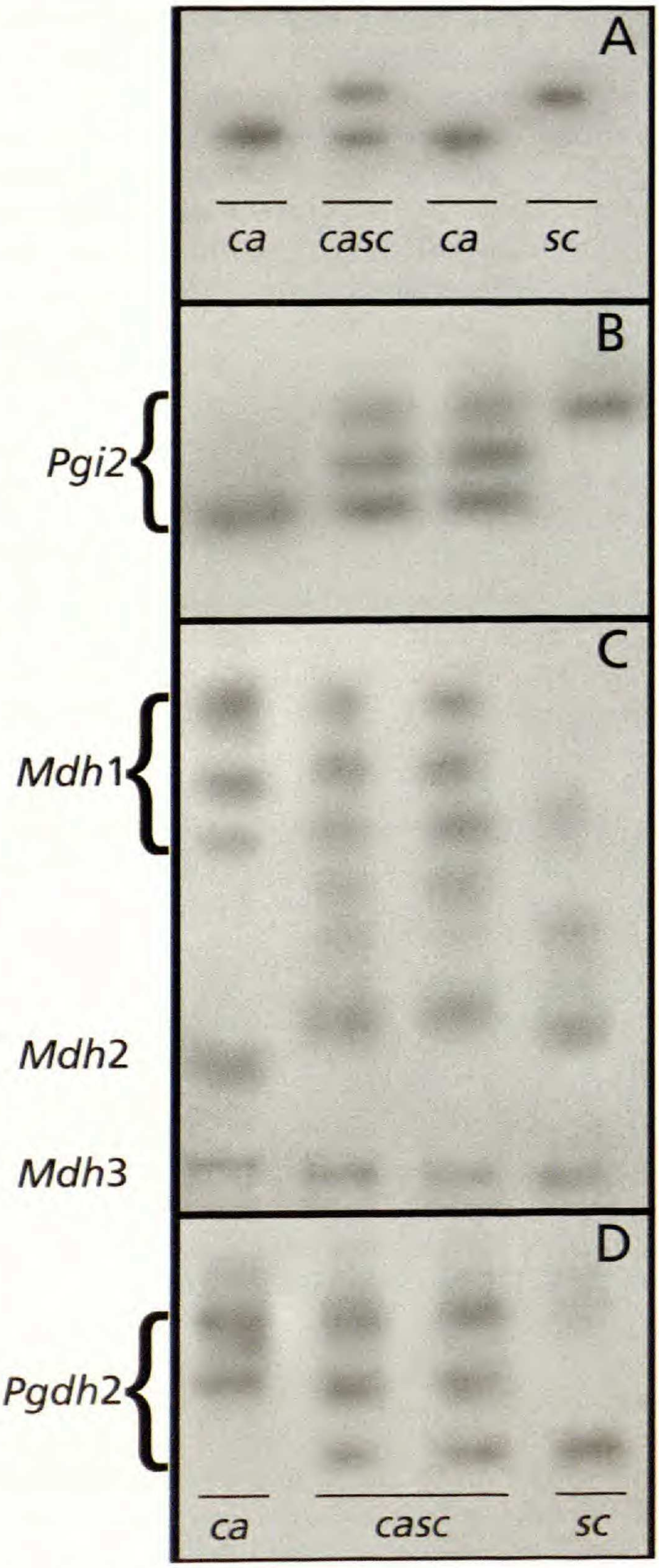

FIG. 4. Representative gels stained for enzymes from *Polypodium* samples included in the present study. *P. calirhiza* = ca; *P. calirhiza* × *scouleri* = casc; *P. scouleri* = sc. A. hexokinase (HK); B. phosphoglucoisomerase (PGI); C. malate dehydrogenase (MDH); and D. phosphogluconate dehydrogenase (PGDH). Sampling in B. & C. corresponds to species as labeled in D. See text for interpretation of banding patterns.

stained for enzymes HK, PGI, PGDH, and MDH combine and confirm parental contributions from the *P. scouleri* and *P. calirhiza* genomes to the hybrid.

The difficulty encountered in developing equivalently distinctive morphological characterizations of the hybrid individuals requires further discussion. Hybrids are often recognized initially because they have morphological peculiarities that can signal the amalgamation of two distinct genomes. Wagner (1962) reviewed deviations from morphological symmetry and their role as indicators of hybrid origins in ferns. For genera studied (*Asplenium*, *Cystopteris*, *Cheilanthes*, *Osmunda*, *Polystichum*, *Pteris*, *Woodsia*), he developed three broad conclusions regarding hybrid morphology: First, hybrid structures form symmetrically, but blend traits from parental lineages. If large differences occur between parental lineages, hybrids tend toward irregular or asymmetric development. Second, when asymmetric development does occur, it is retained in hybrids, and may be useful in identifying the hybrid individual. Third, the discovery of morphological irregularities should key investigators to the possibility of hybrid origins and further study of possible parental lineages. Thirty years of further investigation of these fern genera (e.g., Moran, 1982; Murakami *et al.*, 1999; Yatskievych *et al.*, 1988; Mickel, 1979; Haufler *et al.*, 1990), in addition to other pteridophytes (e.g., Montgomery, 1982; Palmer, 1998; Pryer and Haufler, 1993; Tyron, 1968) support the conclusions on hybrid morphology summarized by Wagner. Likewise, our study reported intermediate, blended traits in *P. calirhiza* × *scouleri* that are consistently expressed in all leaves, and that help to resolve the definition of the hybrid and the identification of parental species. In comparison, other character states were not intermediate between parental lineages, or were so highly variable that they could not contribute to the definition of *P. calirhiza* × *scouleri* (Table 2).

The loss of reticulate venation found in *Asplenium* and *Polystichum* (Wagner, 1962), and other *Polypodium* hybrids (Whitmore and Smith, 1991) is also observed in *P. calirhiza* × *scouleri*. Whitmore and Smith (1991) investigated other members of the western *P. vulgare* complex, and revealed a loss of vein anastomosis following hybridization. They observed only 0–33% of the veins per pinna in *Polypodium calirhiza* anastomose, whereas parental species *P. californicum* has weakly to fully anastomosing venation (5–100%) and *P. glycyrrhiza* produces pinna with entirely free venation. The primarily free venation occurring in *P. calirhiza* × *scouleri* provides further evidence for a propensity toward less reticulated venation whenever genomes differing in this character are present.

Position of fertile pinnae on the leaf (terminal, mid-, lower), often a combination of parental features in fern hybrids (e.g., *Osmunda* hybrids, Wagner, 1962), was not a useful diagnostic character for *P. calirhiza* × *scouleri*. *Polypodium scouleri* and *P. calirhiza* fertile segments tend to be positioned terminally in the former, and may comprise all but the lower 1–3 segment pairs in the latter. Nonetheless, large variation occurs on parental leaves, particularly in *P. calirhiza*. Likewise, hybrid leaves show great variation ranging from few, often terminal segments with sori, to all segments producing sori.

However, the large variation in soral maturation on segments aptly indicates hybridization, with sori found in all developmental stages, albeit with malformed spores.

Fertile vein development and soral position frequently aid in delimiting fern hybrids. For example, *Polystichum lonchitis* produces fertile veins progressing from the midrib to the margin with sori midway and "dorsal" upon the vein whereas *P. acrostichoides* has fertile veins terminating at sori halfway between the margin and the midrib. A combination of parental traits occurs in fertile vein development of the hybrid *P. acrostichoides* × *lonchitis* with some sori terminal on fertile veins, other sori dorsal, and some sori lacking development (Wagner, 1962). In contrast, no distinction in soral position between *P. scouleri* and *P. calirhiza*, as well as the hybrid (when it occurs), is observed. Sori are terminal on fertile veins of parental species and *P. calirhiza* × *scouleri*. Close observation of parental species failed to determine differences in the manner by which fertile veins terminated, and, although *P. calirhiza* veins appear to end in a more reduced and less club-like form than those of *P. scouleri*, this difference may merely result from differences in leaf texture. Leathery hybrid leaves produce fertile veins more closely resembling *P. scouleri*, but, again, this may be an artifact of similar leaf textures. Sori do occur closest to the costa in *P. scouleri* whereas they are located midway between the costa and margin on *P. calirhiza* pinnae. The hybrid is highly variable producing sori both near the costa or midway between it and the pinna margin.

Leaf outlines of hybrids often blend those of parental individuals (Wagner, 1962), but this morphological feature is not transitional in *P. calirhiza* × *scouleri* leaves. Texture, leaf outline, pinna width and apex, as well as sinus angle and depth are all commonly useful for hybrid identification, but are not useful in delimiting *P. calirhiza* × *scouleri*. The resemblance of hybrid leaves to *P. scouleri* may be the most significant factor contributing to past difficulties in recognizing hybrid populations.

An unexpected discovery of the present study regards the average spore length for sampled *P. scouleri* plants from California. Published average spore length (Haufler *et al.*, 1993) for *P. scouleri* is less than 53 μm, whereas *P. calirhiza* spores exceed 58 μm. Indeed, *P. calirhiza* spores from Tank Hill and Mt. Sutro measured well within expected values whereas *P. scouleri* spores exceeded the expected average (Fig. 3). In an effort to explain the increased spore size, *P. scouleri* spores were harvested and measured from plants in Oregon (Hildebrand #3214, KANU), and, with an average of 55.3 μm, fell within the expected range.

Three explanations may account for the larger than average spores obtained from the California *P. scouleri* plants. Certainly, contamination of spore samples may have occurred. Although spores were removed directly from sporangia, *P. calirhiza* spores from dried material may have contaminated the *P. scouleri* plants measured. Two alternative possibilities are more difficult to assess. Environmental factors could account for the increase in average spore length found in California populations of *P. scouleri*. Spore

sizes of *Isoetes* species have been found to be strongly affected by environmental parameters including temperature, solar radiation, and elevation (Cox and Hickey, 1984). Finally, the increased average spore length observed in plants of *P. scouleri* from California could be explained by an increase in chromosome number. Although not always correlated to ploidy level, increases in spore size may indicate the formation of both auto- and allopolyploids. For example, average spore length was found to increase with ploidy level by a multiplier of 1.26 in species of neotropical *Polystichum* (Barrington *et al.*, 1986). The increase in average spore length for sampled California *P. scouleri* plants (ca. 15%) may correspond to an increased chromosome number via autopolyploidy.

To further explore this possibility, guard cells, often positively correlated with ploidy level (Barrington *et al.*, 1986), were compared between Oregon and California *P. scouleri* plants. Guard cell size was previously determined to predict allopolyploidy in the western *P. vulgare* complex. Barrington *et al.* (1986) found tetraploid *P. calirhiza* guard cells average 1.12 and 1.2 times larger than progenitors *P. californicum* and *P. glycyrrhiza*, respectively. Nevertheless, differences in guard cell length between California and Oregon *P. scouleri* populations were not observed, and decreased the possibility of an autopolyploid event in *P. scouleri* from sampled California populations.

Early cytological investigations by Manton (1951) of three plants identified as *P. scouleri* from Point Reyes, California revealed *n* pairs and *n* univalents at meiosis (vs. typically unpaired chromosomes in triploids) and suggested a closer genetic relationship between *Polypodium* tetraploids and diploids in western North America. It now seems most likely that the California plants Manton investigated were not *P. scouleri*, but *P. calirhiza* × *scouleri*. No cytological study has been completed for *P. scouleri* plants from Mt. Sutro and Tank Hill populations, and efforts to re-locate the Point Reyes hybrid populations have been unsuccessful.

The present study provides morphological and molecular evidence for the hybridization of *Polypodium scouleri* and *P. calirhiza* in California, and helps to secure the placement of the former in the western *P. vulgare* complex of North America. Questions remain regarding the California *P. scouleri* populations that merit further investigation. Future cytological studies of these populations, in conjunction with spore length measurements from fresh material, may aid in clarifying any remaining ploidy level conundrums.

Polypodium calirhiza × *scouleri*

Stem stout (5–15 mm diameter), occasionally whitish, acrid to slightly sweet-tasting. Rhizome scales uniformly brown to weakly bicolored with pale margins, lanceolate to lanceolate-ovate, symmetric, with occasional teeth on erose margins. Blades to 39 cm in length with a stout petiole to 3 mm in diameter. Lamina stiff and leathery, ovate-lanceolate, pinnatifid, usually widest at or just above the base, to 20 cm wide; sparsely scaly to

abaxially glabrescent; rachis sparsely puberulent adaxially. Rachis scales, concolorous, pale reddish brown, lanceolate-ovate; often adjacent, fused (from the base) to one third their length. Segments oblong to linear, usually more than 12 mm wide, with rounded apices, sparsely crenulate margins, midribs adaxially glabrous. Venation primarily free but with some anastomoses and irregularly formed aeroles. Sori oval to circular, but with widely varying development, usually closer to midrib than margins, 1–4 mm in diameter, producing malformed spores. Sporangiasters absent.

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